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Rac1 supports muscle glucose uptake independently of Akt

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Insulin plays a major role in the regulation of glucose homeostasis. Insulin-stimulated glucose uptake in skeletal muscle is achieved by increased translocation of intracellular stored GLUT4 glucose transporters to the plasma membrane through the phosphatidylinositol-3 kinase (PI3K) and Akt signalling pathway (Tokarz *et al.* 2018). Furthermore, downstream of PI3K, and in parallel with Akt activation, PI3K also phosphorylates/activates the Rho-family GTPase Rac1, which promotes GLUT4 translocation by initiating cortical actin filaments remodelling (Tokarz *et al.* 2018). Rac1 plays an important role in the reorganization of the actin cytoskeleton and is also essential for insulin-stimulated glucose transport. This reorganization of the cytoskeleton allows for the translocation of GLUT4 transporters to the plasma membrane, which increases glucose uptake into the cell (Tokarz *et al.* 2018). However, it is important to state, that this has not been shown *in vivo* (Madsen *et al.* 2018). Signalling to and downstream of Akt is often found intact in obese individuals, despite reduced insulin-stimulated glucose uptake (Kim *et al.* 2003). Insulin-stimulated Rac1 activation was recently found to be impaired in obese individuals (Sylow *et al.* 2013). Furthermore, the same group demonstrated earlier that muscle-specific knockout of Rac1 (Rac1 mKO) leads to a decrease in exercise-stimulated GLUT4 translocation (Sylow *et al.* 2016), but it has not been shown whether this is also the case for insulin-mediated glucose uptake in muscle. Therefore, it is crucial to investigate the mechanism behind Rac1 activation and development of insulin resistance.

In a paper published recently in *The Journal of Physiology*, Raun *et al.* (2018) demonstrates that Rac1 in the context of high-fat diet (HFD) for 18 weeks is detrimental to insulin-stimulated muscle glucose uptake independently of Akt signalling. To define the role of Rac1 *in vivo*, the authors used an inducible, Rac1 mKO. Wild-type (WT) littermates fed HFD or standard chow served as controls. To test whole-body insulin sensitivity and insulin-mediated muscle and adipose tissue glucose uptake, a bolus of insulin and radiolabelled 2-deoxyglucose (2-DG) were simultaneously administered. In WT mice, HFD impaired whole-body insulin tolerance due to impaired insulin-stimulated glucose uptake in both tissues with no changes of insulin-stimulated Akt phosphorylation. The adverse effect seen with HFD feeding on glucose metabolism is most likely mediated by impaired Rac1 signalling. This was demonstrated by Rac1 mKO, which decreased the insulin-stimulated glucose uptake in both the standard chow- and HFD-fed mice, despite normal Akt signalling. This provides evidence that knocking down Rac1 impairs insulin tolerance, but it is not clear that a reduction in Rac1 mediates the effects of a HFD. To further confirm the observations seen *in vivo*, muscles (soleus and extensor digitorum longus) from control and Rac1 mKO animals were isolated and incubated with insulin and 2-DG to confirm reductions in insulin-stimulated glucose uptake. The results indicate that the lower insulin-stimulated glucose uptake in HFD Rac1 mKO mice was due to a defect of the transport step across the muscle membrane. Furthermore, because blood circulation is removed in the incubation, the delivery of glucose to the muscle through the blood is prevented, which is a limiting factor. Importantly, muscle glycogen content was not affected by Rac1 mKO, suggesting that Rac1 does not regulate glycogen synthesis. The adverse effect of Rac1 mKO in HFD-fed mice on whole-body insulin tolerance was partially compensated by increased insulin-stimulated phosphorylation of Thr308 and Ser473 residues of Akt and glucose uptake in adipose tissue. Also, the Rac1 protein content was increased in the adipose tissue of the Rac1 mKO fed HFD, which may explain the increase in glucose uptake.

Some limitations in this study can be addressed by several future studies that look at muscle fractionation and immunoblotting for GLUT4 content, to confirm that this was indeed the mechanism by which altering Rac1 signalling altered glucose uptake, and to investigate the messenger responsible for the cross talk between adipose and muscle tissues. Various myokines, adipokines, cytokines and other chemical messengers in the blood could be important mediators for the cross-talk between skeletal muscle and adipose tissue, which makes investigating blood plasma for these chemical messengers important. This study proved that when Rac1 is knocked out in muscle tissue, Akt phosphorylation was upregulated in adipose tissue, which may be a reason why there was not a complete absence of glucose uptake in the mice. In order to investigate this phenomenon, a future study could examine adipose tissue-specific Rac1 knockouts, to investigate whether or not muscle tissue is able to compensate and adapt like adipose tissue. In order to investigate this phenomenon even further, Rac1 could be knocked out in both muscle and adipose tissue in the same animal model to see if the effect on glucose uptake is worse than when Rac1 content is decreased in just one of the tissues. This is important because if Rac1 is knocked out in both tissues and glucose uptake is still functioning, then there are other key players and or mechanism responsible for the regulation of glucose uptake in these tissues. Another mechanism that can be employed to investigate the cross talk between skeletal muscle and adipose tissue is by transplanting adipose tissue from Rac1 mKO mice into insulin-resistant mice to see if it corrects whole-body glucose tolerance. Furthermore, white adipose tissue with increased Rac1 content could also be transplanted to observe the effects on whole-body metabolism of the receiving mouse. Another technique to investigate Rac1 and its role in adipose tissue is to overexpress it in adipose tissue in order to investigate the effects on diet-induced insulin resistance.

In this paper, the authors provide several important new insights on how obesity is associated with insulin resistance and how the lack of Rac1 exacerbates the detrimental effect of HFD and reduces

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whole-body insulin action due to reduced insulin-stimulated glucose uptake in skeletal muscles. The data indicate that Rac1 signalling is important for insulin-mediated glucose uptake. This paper also highlights the under-appreciated role of adipose tissue in mediating whole-body glucose disposal. In addition, Rac1 has other known functions (e.g. PAK1 signalling and NOX2 activation), so future studies need to pinpoint the direct mechanism in which loss of Rac1 leads to decreased glucose uptake during insulin stimulation in muscle.

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Additional information

Competing interests

None

Author contributions

Both authors have read and approved the final version of this manuscript and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

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